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STRUCTURE AND FUNCTION OF NATURAL KILLER CELL SURFACE RECEPTORS*

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Key Words KIR, KIR/HLA complex, allotype specificity, CD94, Ly49A/H-2D^d complex

■ **Abstract** Since mid-1990, with cloning and identification of several families of natural killer (NK) receptors, research on NK cells began to receive appreciable attention. Determination of structures of NK cell surface receptors and their ligand complexes led to a fast growth in our understanding of the activation and ligand recognition by these receptors as well as their function in innate immunity. Functionally, NK cell surface receptors are divided into two groups, the inhibitory and the activating receptors. Structurally, they belong to either the immunoglobulin (Ig)-like receptor superfamily or the C-type lectin-like receptor (CTLR) superfamily. Their ligands are either members of class I major histocompatibility complexes (MHC) or homologs of class I MHC molecules. The inhibitory form of NK receptors provides the protective immunity through recognizing class I MHC molecules with self-peptides on healthy host cells. The activating, or the noninhibitory, NK receptors mediate the killing of tumor or virally infected cells through their specific ligand recognition. The structures of activating and inhibitory NK cell surface receptors and their complexes with the ligands determined to date, including killer immunoglobulin-like receptors (KIRs) and their complexes with HLA molecules, CD94, Ly49A, and its complex with H-2D^d, and NKG2D receptors and their complexes with class I MHC homologs, are reviewed here.

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INTRODUCTION

As part of innate immunity, natural killer (NK) cells are capable of killing certain tumor and virally infected cells. A balance between the activating and inhibitory receptors on the cells surface controls the cytolytic activity of NK cells. The inhibitory receptors suppress NK cell lysis of target cells that express class I MHC molecules, but allow the lysis of class I negative cells. Structurally, inhibitory receptors belong to either the immunoglobulin-like superfamily (IgSF) or the C-type lectin-like receptor (CTLR) superfamily. IgSF inhibitory receptors include the human killer cell Ig-like receptors (KIR), which recognize the $\alpha 1$ and $\alpha 2$ domains of human leukocyte antigens (HLA-A, -B, and -C) with the bound self-peptide, and the Ig-like transcripts (ILTs, also named as leukocyte Ig-like receptors or LIRs) expressed on myeloid cells that recognize the nonpolymorphic $\alpha 3$ domain of classical and nonclassical HLA molecules (38). Members of CTLR superfamily include the CD94/NKG2A that recognize the nonclassical class I molecules HLA-E and Qa-1 in human and mouse (71), respectively, and the murine Ly49 molecules that recognize the classical class I MHC molecules (44, 71). All inhibitory receptors carry immunotyrosine inhibitory motifs (ITIM) in their cytoplasmic domain. The Ly49 receptors are found only in mice and appear to be functional orthologs to KIR, which are found in primates but not in rodents (33). The crystal structures of three KIRs (22, 46, 67), one ILT receptor, and a CD94 homodimer have been published (6, 9). Examples of MHC recognition by KIR and Ly49 inhibitory receptors were shown in the cocrystal structures of KIR2DL2/HLA-Cw3, KIR2DL1/HLA-Cw4, and Ly49A/H2-D^d (5, 21, 70).

Like inhibitory receptors, activating receptors are either IgSF or CTLR superfamily members. Activating IgSF receptors include 2B4, the natural cytotoxicity receptors NKp46, NKp30, and NKp44 (54), the noninhibitory isoforms of KIRs, and ILTs/LIRs. Activating CTLR include CD94/NKG2C and CD94/NKG2E heterodimers, NKG2D homodimer, and activating isoforms of Ly49. All activating receptors lack activating motifs in their cytoplasmic domains. However, all of them,

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except 2B4, display a charged lysine or arginine residues in their transmembrane region to pair with a negatively charged residue on adaptor molecules, such as DAP12, DAP10, CD3ζ, or FcRγ, that carry signal transduction components.

In contrast to the inhibitory receptors much less is known about the structures of activating NK receptors. The only structure of the activating NK receptor determined to date is that of NKG2D (77). It is a member of CTLR superfamily and is distantly related to NKG2A, B, C, and E. NKG2D mediates NK cells cytotoxicity against certain tumor cells and provides costimulatory signals on $CD8^+$ $\alpha\beta$ and $\gamma\delta$ T cells against virally infected cells (2, 15, 28, 72, 78). Stress-induced molecules MIC-A, MIC-B, and ULBPs in human, Rae-1, and H-60 in mice have been identified as the ligands for NKG2D (2, 8, 17). Recently, the crystal structures of a murine NKG2D in complex with Rae-1β and a human NKG2D in complex with both MIC-A and ULBP3 have been determined (40, 41, 60).

THE KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR SUPERFAMILY

Approximately 12 genes sharing greater than 90% sequence identity encode human KIRs. They are located in the leukocyte receptor complex (LRC) region on chromosome 19q (68, 76). A similar number of KIR genes have been identified in other primate species (34). In addition, several homologous receptor families, including immunoglobulin-like transcripts (ILT) or leukocyte immunoglobulin-like receptors (LIR) (4, 11, 63), leukocyte-associated Ig-like receptors (LAIR) (50), paired Ig-like receptors (PIR), and gp49 (1), have also been identified (30, 36). They display 35%–50% sequence identity and clearly share a common fold with KIR. Together, they define a so-called KIR superfamily. A more distantly related set of proteins are the Ig-like Fc receptors (FcαR, FcγR-I, -IIa, -IIb, -III, and FcεRI), which display less than 20% sequence identity with KIR but nonetheless share a structural fold similar to it.

KIRs are type I transmembrane glycoproteins with two or three extracellular C2-type Ig-like domains (12, 14, 73). ILTs contain two or four Ig-like domains; LAIR-1 and -2 contain a single Ig-like domain; PIR-A and -B each contain six Ig-like domains. KIRs exist as either inhibitory or noninhibitory forms (20, 43, 55). The inhibitory forms of these receptors possess ITIM in their cytoplasmic tail. The noninhibitory forms of KIR have a shorter cytoplasmic tail and display a positively charged residue in their transmembrane regions through which they pair with an activating motif-containing adaptor molecule (38, 44).

The Structure of KIR

To date, the crystal structures of the extracellular domains of three members of KIR family, KIR2DL1, KIR2DL2 and KIR2DL3, have been published (22, 46, 67). Overall, the KIR fold is similar to the C2-type Ig-like fold observed in the

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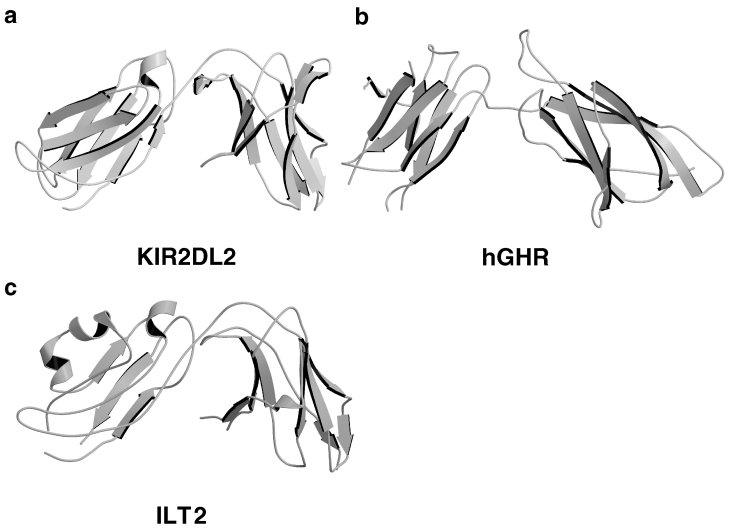

Figure 1 Comparison of KIRs with other representative two-domain Ig-like receptors. (*a*) KIR2DL2, (*b*) hGHR, (*c*) ILT-2. PDB codes: KIR2DL2, 1EFX; hGHR, 3HHR; ILT2, 1G0X.

hematopoietic receptors with the difference existing primarily in the pairing of two β strands (Figure 1). In hematopoietic receptors, β strand A pairs with strand B, whereas in KIR structures the first strand splits into two strands, A and A′, which hydrogen-bond with the B and G strands, respectively. This results in a "strand switching" in KIR that is likely attributable to the presence of a conserved *cis*-proline residue in the first strand. In addition, KIR structures possess unique tertiary packing. In particular, the hinge angle between the N-terminal D1 and C-terminal D2 domains is smaller than those observed in other two-Ig-like domain structures, such as human growth hormone receptor, the V and C domains of T cell antigen receptors, and the V and C_{H1} domains of antibodies. The hinge angle of KIR varies from 66° in KIR2DL1 to 81° in KIR2DL2 and KIR2DL3. This domain hinge angle is stabilized by a highly conserved interdomain hydrophobic core (hinge core) that consists of Leu17, Met69, Val100, Ile101, Thr102, His138, Phe178, Ser180, Pro185, Tyr186, and Trp188. An interdomain salt bridge between Asp98 and Arg149, conserved in all KIR family sequences, also helps restrict the hinge angle. Aside from the differences in the hinge angle, the structures of KIR2DL1, KIR2DL2, and KIR2DL3 are nearly identical with rms deviations less than 1 Å between the Cα atoms of their respective domains.

The Structure of LIR-1 (ILT-2)

In addition to KIR, the structure of the first two domains of LIR-1 (ILT-2), an inhibitory receptor expressed on monocytes, B cells, dendritic cells, and subsets

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of NK and T cells, has also been determined (9). Overall, the sequence homologies between KIR and ILT genes are about 40%, and domains 1 and 2 of LIR-1 possess the KIR type Ig fold including strand switching in the first β strands. However, distinct structural differences are observed between LIR-1 and KIRs (9). In particular, LIR-1 has two unique short 3_{10} helices in each domain. One replaces the C′ strand in the D1 domain and the C-terminal end of the C′ strand in the D2 domain found in KIR, and the other is situated between the E and F strands in the D1 domain and between the F and G strands of the D2 domain (Figure 1). A short left-handed type II polyproline-like helix is also found in the F-G loop of the D1 and D2 domains. Like KIR, primarily hydrophobic residues forming a hinge core that stabilizes the D1-D2 interdomain conformation occupy the interdomain region of LIR-1. Interestingly, a conserved interdomain salt bridge in KIR between Asp98 and Arg149 is absent in the LIR-1 structure, possibly contributing to the slightly larger hinge angle of LIR-1 (88°).

RECOGNITION OF CLASS I MHC LIGANDS BY KIR

The Structure of KIR/HLA Complexes

Class I MHC molecules were first implicated as potential ligands of NK cell receptors when an inverse relationship was identified between the susceptibility of target cells to NK cell-mediated lysis and the expression of class I on target cells (29, 69). It was further demonstrated that transfection of class I genes into a class I–deficient target cell was sufficient to protect these cells from NK cell-mediated lysis (64). Evidence for the involvement of multiple receptors that recognized distinct HLA class I molecules came from studies examining the specificity of cloned NK cells against different allogenic target cells. This led to the identification of two KIR molecules, KIR2DL1 (CD158a) and KIR2DL2 (CD158b1), that recognize HLA-Cw2, 4, 6, 15, and HLA-Cw1, 3, 7, 8 allotypes of class I MHC (12, 53, 73), respectively. Sequence comparison of HLA ligands suggested residues 77 and 80 in class I MHC heavy chains are important for their receptor specificities (10).

The first crystal structure of the KIR/HLA complex was solved between KIR2DL2 and HLA-Cw3 with a nonamer self-peptide GAVDPLLAL (GAV) derived from the importin-α1 subunit (5). More recently, a related structure of KIR2DL1/HLA-Cw4 with a bound peptide QYDDAVYKL (QYD) was determined (21). The orientation of KIR with respect to HLA is similar to that of TCRs, with the D1 and D2 domains of KIR assuming the respective positions of the Vα and Vβ domains of TCR (19) (Figure 2). The footprint of KIR on the class I HLA overlaps partially with those of TCRs on class I molecules. However, KIR contacts primarily the P7 and P8 positions of the bound peptide and associated HLA residues, whereas the TCR interacts predominantly with the P4, P5, and P6 positions of the peptide (19, 24, 74).

The KIR/HLA complex buries approximately 1600 Å^2 surface area, similar to TCR/MHC complex. Unlike TCR/MHC interfaces that consist largely of hydrogen

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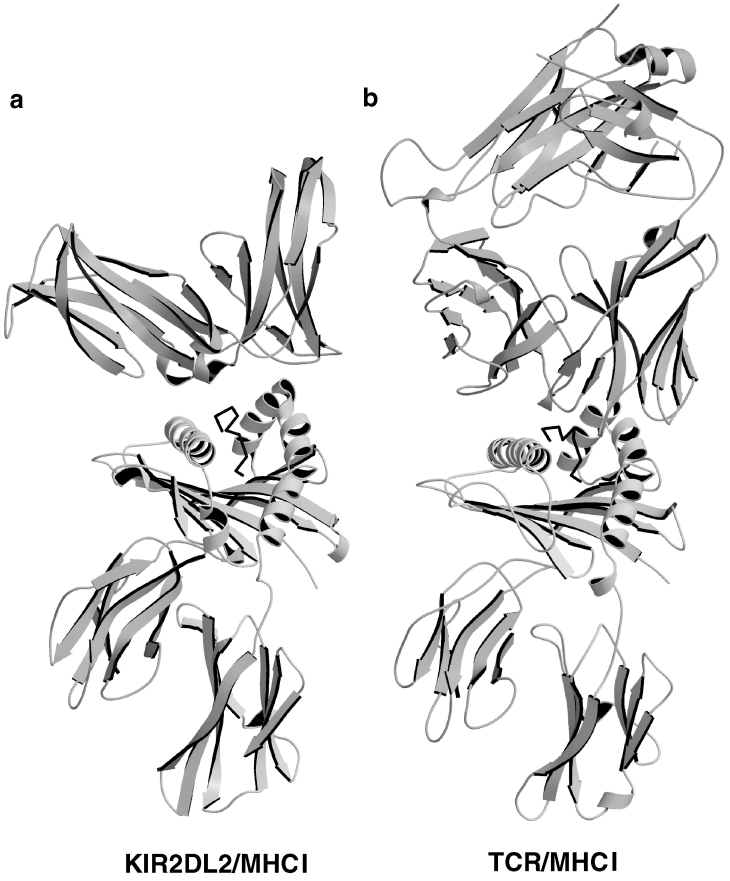

Figure 2 Comparison of KIR/MHC (PDB code 1EFX) binding to TCR/MHC (PDB code 1BD2) binding.

bonds and hydrophobic and van der Waals interactions, the KIR/HLA interface is characterized by strong charge complementarity. In all, there are six acidic residues in KIR that interact with six basic residues in HLA-Cw3, resulting in the formation of four salt bridges between E21, E106, D135, D183 of KIR and R69, R151, R145, K146 of HLA-Cw3, respectively. The dominance of charge-charge interactions in the interface resembles the interface between adhesion receptors such as CD2/CD58 (75). To evaluate the contribution of these salt bridges to the KIR/HLA recognition, three single mutations of the receptor, E106A, D135H, and D183A, were created to remove three of the four salt bridges individually, and their effects on HLA binding were measured by surface plasmon resonance (SPR). While the wild-type receptor binds HLA-Cw3 (GAV) with 30 μM affinity, E106A resulted in a sixfold reduction in the ligand binding affinity, and both D135H and

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D183A mutant showed no detectable binding to the ligand. In addition to charge complementarity, the KIR/HLA interface also displays a network of six hydrogen bonds and hydrophobic interactions. The largest hydrophobic cluster includes the aliphatic portions of K44, F45, M70, and Q71 of KIR2DL2 together with the aliphatic portions of V76, R69, R75, and R79 of HLA-Cw3.

Class I Allotypic Recognition of KIR

KIRs are known to recognize multiple alleles of MHC molecules. For example, KIR2DL1 recognizes HLA-Cw2, 4, 5, 6, and 15, whereas KIR2DL2 and KIR2DL3 recognize HLA-Cw1, 3, 7, and 8 allotypes. The basis of this receptor recognition of allotypic HLA molecules was not understood until the structure of the KIR/HLA complex was solved. Among the 12 HLA-Cw3 interface residues, 11 are invariant across all HLA-C alleles despite their location within the polymorphic region of the class I heavy chain. Amino acid 80 is the only variable residue contributing to the receptor interface. In contrast, 8 of 16 HLA-A2 residues in contact with the A6 TCR are variable among the HLA-A alleles. The use of highly conserved residues within an otherwise polymorphic region of HLA enables individual KIR to recognize multiple class I HLA molecules while discriminating among various allotypes based on the identity of amino acid position 80. The recognition of conserved HLA residues by KIR has important ramifications and may reflect the functional differences between the innate and adaptive immune systems. TCRs rely on gene rearrangement and on positive selection of T cell clones to achieve exquisite specificity for specific peptides complexed to polymorphic MHC molecules. This combination of TCR maturation and the polymorphism in MHC molecules enables T cell–mediated cellular immunity to counteract virtually unlimited forms of pathogens. KIRs, as part of the innate immunity, are obligated to produce a rapid response. These receptors are germline encoded, thus they have limited ability to adapt to the evolving peptide-MHC diversities. In addition, there are significantly more MHC alleles than there are KIRs. As a result, to provide effective surveillance of MHCs by KIR requires individual KIR to recognize more than one MHC allele. By focusing recognition on conserved residues within the polymorphic regions of MHC, distinct KIR can recognize multiple related MHC molecules. Through the use of the conserved residues for KIR recognition and polymorphic residues for TCR recognition, a given class I MHC effectively accommodates the requirements for recognition by both the innate and adaptive immune systems.

As mentioned earlier, a single HLA residue at position 80 determines KIR allotype specificity. From the receptor side a single KIR residue at position 44 controls exquisite allotype specificity toward HLA. For example, KIR2DL1 with a Met at position 44 recognizes HLA-Cw2, 4, 5, 6, and 15 allotypes with Lys at position 80. KIR2DL2 has a Lys at position 44 and does not recognize HLA-C molecules with a Lys at position 80. Instead, it recognizes HLA-Cw1, 3, 7, and 8 allotypes with Asn at position 80 (Figure 3). In the crystal structure of the KIR/HLA complex, Lys44 of KIR2DL2 makes a hydrogen bond with Asn80 of HLA-Cw3. Replacing Asn80 with Lys, as in the sequence of HLA-Cw2, 4, 5, and

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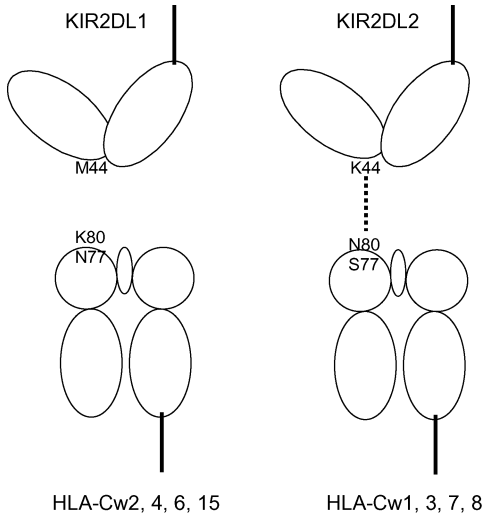

Figure 3 Schematic diagram illustrating the allotype specificity of KIR2D receptors. The *dotted line* represents the hydrogen bond between Lys44 of KIR2DL2 and Asn80 of HLA-Cw3.

6, would generate an unfavorable electrostatic interaction with Lys44 of KIR2DL2. Similarly, replacing Lys44 of KIR2DL2 with Met would result in the loss of the (KIR)Lys44-Asn80 (HLA) hydrogen bond and destabilize the KIR/HLA interface.

The Peptide Preference for KIR/HLA Binding

Peptides are involved not only in the generation of stable, properly folded MHC molecules at the cell surface but also in the direct recognition of class I MHC by NK cells. TAP-deficient cells cultured at 26°C in the absence of class I binding peptides, express "empty" class I MHC molecules on their cell surface. These class I molecules are unable to protect the target cells from NK-mediated lysis. Peptide preferences were observed in KIR2D recognition of HLA-C molecules (61, 80). The crystal structure of the KIR2DL2/HLA-Cw3 complex contains a nonamer peptide GAVDPLLAL (GAV) derived from the human importin-α1 subunit (80). KIR2DL2 makes direct contacts to the GAV peptide at both the P7 and P8 positions. The side chain of P7 leucine makes loose contacts with Leu104 and Tyr105 of the KIR. At the P8 position, Gln71 of KIR forms a hydrogen bond with the backbone amide nitrogen of the peptide. Compared to T cell receptor-MHC recognition, KIR makes less contact to the side chain of the peptide and thus is less sensitive to the peptide content. The formation of this backbone hydrogen bond may restrain the size of the P8 side chain owing to steric hindrance with the receptor. Indeed, when substitutions were introduced at the P8 position of GAV peptide the results showed that amino acids larger than Val at the P8 position completely abolished the receptor binding.

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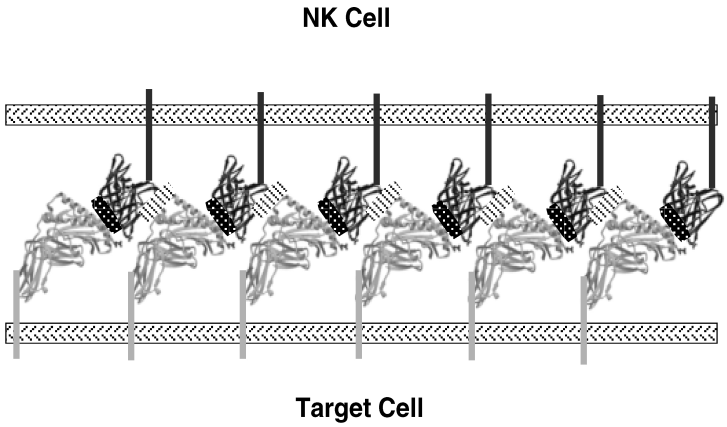

Figure 4 A lattice model for HLA-induced KIR clustering.

A Model for KIR/HLA Aggregation

Ligand-induced receptor oligomerization is presumed to be a common mechanism for initiating receptor-mediated signaling. The so-called immunological synapses at the interface of T cells and APC consist of a central cluster of TCR/MHC complexes and a peripheral ring of adhesion molecules (27, 52). In contrast, the observed NK cell immune synapse is formed with a central cluster of adhesion molecules LFA-1/ICAM-1 and a peripheral KIR/HLA cluster in the shape of a donut (16). The question of how the KIR/MHC complex forms this ordered donut-shaped oligomeric aggregate remains unclear. One possible form of oligomerization was observed in crystals of the KIR2DL2/HLA-Cw3 complex. Within these crystals, each KIR molecule makes an additional contact, apart from the functional binding interface, with a symmetry-related HLA-Cw3 molecule in a peptide-independent manner. The contact buries 530 $Å^2$ of surface area and is characterized by mostly van der Waals interactions. Interestingly, this KIR/HLA contact bridges between the adjacent pair of complexes into an oligomeric KIR/HLA aggregate (Figure 4). In this form of oligomer, the KIR/HLA complexes are all in the same orientation, and the molar ratio between receptor and ligand is maintained at 1:1. Furthermore, the putative glycosylation sites on both KIR2DL2 and HLA-Cw3 are away from the oligomerization interface. It is possible that this form of receptor-ligand oligomerization resembles the receptor clustering on the surface of NK cells.

C-TYPE LECTIN-LIKE NK RECEPTORS

The Structure of CD94 and Ly49 Receptors

C-type lectins are a family of calcium-dependent carbohydrate binding proteins, such as the mannose binding protein (MBP), E-selectins, tetranectins, and

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lithostathines. The carbohydrate binding domains (CRD) are approximately 120 amino acids and contain three disulfide bonds and a characteristic Ca^{2+} binding loop that is essential for carbohydrate binding. Recently, many cell surface receptor proteins have been identified that contain the homologous CRD sequences, including the conserved cysteine residues. Some of them, however, do not appear to have the conserved calcium binding residues. Collectively, these receptors are referred to as members of the C-type lectin-like receptor (CTLR) superfamily. CD94 and members of the NKG2 and Ly49 family of receptors are part of the CTLR superfamily.

The structure of CD94 showed that the putative CRD domain folds into a C-type lectin fold (6) (Figure 5). There are, however, distinct features separating CD94 from classic C-type lectins. First, the putative Ca^{2+} binding loop in CD94 lacks the conserved calcium-chelating aspartate and glutamate residues and is in a different conformation than other Ca^{2+} binding loops observed in classic C-type lectins. It is therefore predicted that the function of CD94 does not require the binding of carbohydrate. Second, there are two canonical α helices in the structure of C-lectins. Only one is observed in the structure of CD94. The residues at the position of the second helix have adopted a loop conformation at the CD94 dimer interface. The dimer of CD94 is stabilized primarily by hydrophobic interactions

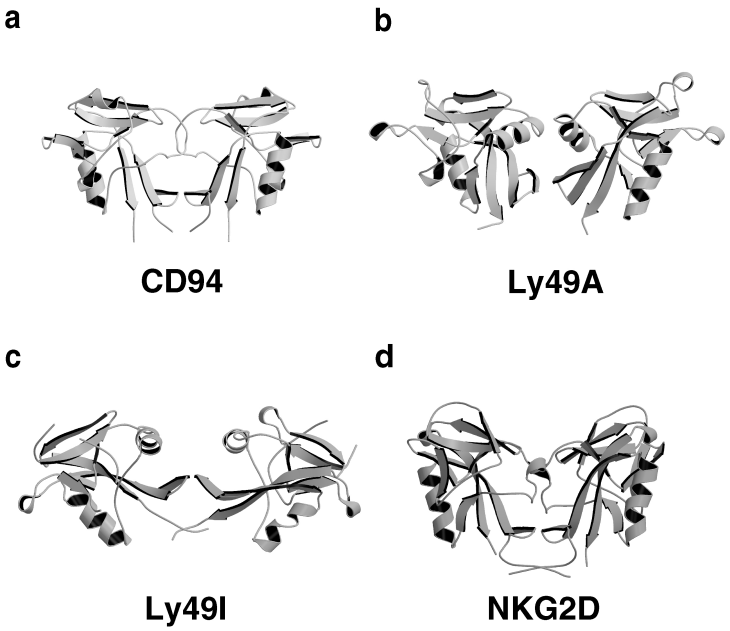

Figure 5 Structures of members of CTLR superfamily. Shown here are dimmers of (*a*) CD94 (PDB code 1B6E), (*b*) Ly49A (PDB code 1QO3), (*c*) Ly49I (PDB code 1JA3), and (*d*) NKG2D (PDB code 1KCG).

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and buries about 1200 Å^2 of interface area. More recently, the crystal structure of NKG2D provided another example of the receptors from the CTLR superfamily (77). The fold of NKG2D is similar to that of CD94, including the deformed second helix. NKG2D exists as a functional homodimer whose structure can be readily superimposed onto the dimer structure of CD94.

In contrast, the structure of the Ly49A monomer showed that the murine receptor assumes a fold more similar to that of the mannose binding protein (MBP) than to that of CD94 or NKG2D (41, 70) (Figure 5). In particular, the structure of Ly49 preserves the second α helix of MBP, whereas the same region adopts a loop conformation in the structure of CD94. However, the structure of the Ly49A dimer is more similar to the structure of the CD94 homodimer than to that of the MBP. More recently, the crystal structure of Ly49I has been determined (18) (Figure 5). Overall structure of the Ly49I monomer resembles that of Ly49A. Due to the truncation in the expression construct, the crystallized Ly49I fragment existed as a monomer rather than as a dimer in solution. A noncrystallographic dimer was observed in Ly49I crystal that buries 1600 Å^2 surface area. The mode of dimerization as observed in Ly49I crystal, however, is different from that observed in Ly49A, CD94, and NKG2D.

The Structure of NKG2D

The NKG2 gene family located on human chromosome 12p13.1 encodes several similar type II lectin-like proteins known as NKG2A, B, C, D, E, and F (25, 59). NKG2A, B, C, and E show 94%–95% amino acid homology in their extracellular domain and 56% homology throughout the internal and transmembrane regions (32). NKG2D is distantly related to other members of NKG2 family and shows only 21% overall homology to them. All NKG2 proteins except NKG2D form heterodimers with C-type lectin-like receptor CD94 (39). In contrast NKG2D forms a homodimeric structure. Recently, NKG2D generated tremendous interest owing to the finding that it activates NK cells upon the recognition of stress-inducible MIC-A, which is frequently expressed in epithelial tumors (2). NKG2D can also be found on most $\gamma\delta$ T cells, CD8+ $\alpha\beta$ T cells, and macrophages (2, 17). NKG2D associates with a signaling adapter molecule DAP10 through a charged transmembrane residue (78). NKG2D orthologs were also found in other mammals such as chimpanzee, rhesus monkey, cattle, pig, rat, and mice (3, 26, 31, 37, 65, 79).

The crystal structure of extracellular domain of intact murine NKG2D (77) reveals a C-type lectin fold similar to CD94, Ly49A, rat MBP-A, and CD69 (6, 35, 56, 70) (Figure 5). Superposition of NKG2D with CD94 and Ly49A resulted in rms deviations of 1.4 Å and 1.7 Å, respectively, for 97 pairs of Cα atoms. Unlike Ly49A, which retains the intact α2 helix of classical C-type lectins, the α2 helix in NKG2D is deformed into only a one-turn helix. Like CD94, NKG2D also lacks the appropriate Ca^{2+} ligands in its corresponding Ca^{2+} binding loops and thus is presumed to be a non-Ca^{2+} binding C-type lectin-like receptor.

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The NKG2D dimer is similar to the CD94 homodimer. Owing to an extension of the dimer interface by the N termini strands of NKG2D, the interface buries about 1900 $Å^2$, which is substantially more than the 1200 $Å^2$ dimer interface of CD94.

RECOGNITION OF CLASS I MHC BY LY49A

The structure of the C-type lectin domain of Ly49A in complex with H-2D^d is the first and the only example of class I MHC recognition by a murine inhibitory receptor Ly49A (70). In the crystal structure, Ly49A binds H-2D^d at two distinct locations, site 1 and site 2. Site 1 buries ~1000 $Å^2$ surface area and is located at the N-terminal end of the α1 helix of the MHC molecule (Figure 6). This interface is dominated by electrostatic interactions. Site 2 of Ly49A is located under the β sheet floor of the α1 and α2 domains interacting primarily with the α2, α3, and β2m subunits of H-2D^d. It buries more than 3000 $Å^2$ interface area. The interactions between Ly49A and the MHC at site 2 are made primarily by hydrogen bonds. Despite the smaller buried receptor-MHC interface, the orientation of site 1 is consistent with *trans* receptor-ligand recognition, whereas the site 2 appears to be in a *cis* orientation in which both Ly49A and H-2D^d are coming from the target cell. In addition, site 2 involves no polymorphic residues of MHC, making it difficult to explain the allelic specificity of Ly49A. Therefore, site 1 was proposed to be physiologically relevant. The crystal structure of the complex revealed no peptide contact between Ly49A and H-2D^d. This recognition model, although consistent with the nonpeptide dependence of Ly49A recognition, appears contradictory when applied to the MHC recognition by Ly49C. Subsequent mutational analysis of the binding site suggested site 2 is a more likely true binding site (49, 51). Compared to its human counterpart KIR, the recognition mode between Ly49A and H-2D^d is different from that between KIR and HLA, which resembles the TCR/MHC recognition mode.

LIGANDS OF NKG2D

Several NKG2D ligands were recently identified in both humans and mice. MIC-A and MIC-B were among the first identified ligands in humans. Structurally, MIC proteins are described as MHC class I homologs but without the association of β2-microglobulin and peptide (42) (Figure 6). Recently, a group of proteins that bind to the human cytomegalovirus glycoprotein, UL16, has been identified as ligands to human NKG2D and named ULBP. ULBP proteins contain only α1 and α2 domains similar to those of MHC class I, do not bind peptides, and are glycosyl-phosphatidylinositol (GPI) anchored to the membrane (13). In mice the retinoid acid early inducible 1 (Rae-1) family of proteins and H60 protein encoded on murine chromosome 10 were identified as ligands for murine NKG2D

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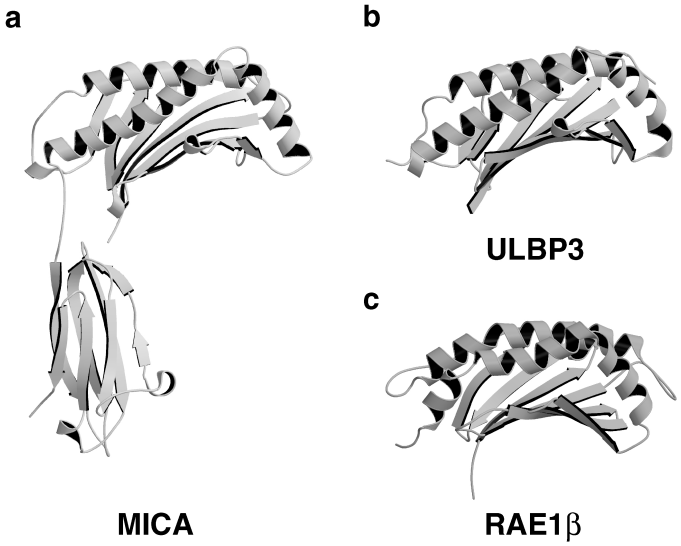

Figure 6 Ligands of NKG2D. The structures of (*a*) MIC-A (PDB code 1HYR), (*b*) ULBP3 (PDB code 1KCG), and (*c*) Rae-1β (PDB code 1JSK).

(8, 17, 47, 57). Like ULBP proteins, Rae-1 and H60 contain only α1 and α2 domains similar to those of class I MHC, do not bind peptides, and are GPI anchored to the membrane (41).

Murine and human NKG2D display significant conservation both at the amino acid sequence and at the three-dimensional structural level. The overall sequence identity of the receptor is about 60% between human and mouse. At the structural level, the superposition between the human and murine NKG2D results in an rms deviation of 0.95 Å among 233 Cα atoms. On the other hand, sequence comparison among NKG2D ligands shows limited identity between them, 24%–33%. That makes NKG2D a unique activating receptor with the ability to bind diverse MHC class I–like ligands.

A structural comparison between MIC-A, ULBP3, and Rae-1β resulted in rms differences of 2.3–2.4 Å between ULBP3 and Rae-1β or MIC-A and 3.9 Å between Rae-1β and MIC-A for 145 Cα atoms. Structural comparison between NKG2D ligands and classical MHC class I molecule HLA-Cw3 results in 2.7–3.4 Å rms differencesfor a similar number of Cα atoms. The conformation of the eight-stranded β sheet agrees quite well among ULBP3, MIC-A, Rae-1β, and HLA-Cw3. The differences exist primarily in the loop and helical regions. All class I/II MHC molecules present peptides, whereas no peptide has been found associating with MIC, ULBP, or Rae-1 (40, 41, 60). In addition, the spacing between the α

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helices of NKG2D ligands is significantly narrower (down to 8–14 Å for ULBP3) than that of HLA-Cw3 (15–20 Å).

LIGAND RECOGNITION BY NKG2D

The crystal structures of NKG2D/MIC-A, NKG2D/ULBP3, and NKG2D/Rae-1β complexes that allow detailed insight into their binding mode were recently determined (40, 41, 60) (Figure 7). In all three complexes the mode of complex formation is similar: NKG2D uses its β strands and loops located at the end opposite to both the N and C termini to bind to the $\alpha1/\alpha2$ helical surface of its ligands.

The relative orientation between NKG2D and its ligands is similar to that between KIR and HLA, and between TCR and their MHC ligands. The long axis of the receptor fits diagonally across the helical axes of a ligand. The receptor footprint covers the C-terminal half of the $\alpha1$ helix and the N-terminal half of the $\alpha3$ helix of a ligand. Both subunits of the NKG2D bind ligand with identical receptor loops. However, the interaction between a homodimeric NKG2D and the asymmetrical ligand results in an asymmetrical receptor subunit orientation that contrasts with the perfect twofold symmetry observed in ligand-free murine receptor.

The two NKG2D subunits form a concave surface in which the convex-shaped ligand interacts with both subunits of the receptor. The interface shape complementarity value Sc is 0.65, 0.72, and 0.63 between the NKG2D and ULBP3, NKG2D and MIC-A, and NKG2D and Rae-1β, respectively, which indicates a good surface complementarity at the receptor-ligand interface. The total buried interface between NKG2D receptors and their ligands is 1900–2200 Å^2, which is larger than the 1560 Å^2 interface between KIR and HLA (5), or the 1700–1800 Å^2 between TCR and MHC (23, 24, 62). Except at the ligand binding interface, no significant conformational changes occurred in the receptor structure upon complex formation. An rms deviation of 0.48 Å among 246 Cα atoms was observed between the structure of a ligand-free and Rae-1β-bound murine NKG2D. Similarly, no large conformational adjustment occurred in the ligand structure upon receptor binding. For the structures of receptor-free and receptor-bound MIC-A and Rae-1β, the rms differences were 0.92 and 0.57 Å, respectively, for 147 Cα pairs.

Each receptor subunit contributes approximately half of the total interface area. Primarily a network of hydrogen bonds and hydrophobic interactions stabilize the receptor-ligand interface. Although the mode of the binding is similar between three different complexes, detailed examination of the binding interface reveals remarkable differences between complexes. When the three NKG2D complexes are superimposed onto each other by using only their receptors, the different ligand orientations become visible. In this case, there is about a 6° orientation difference between ULBP3 and MIC-A, 10° between ULBP3 and Rae-1β, and 10° between MIC-A and Rae-1β. These orientation differences probably reflect local adjustments of the receptor/ligand structures in an attempt to maximize their

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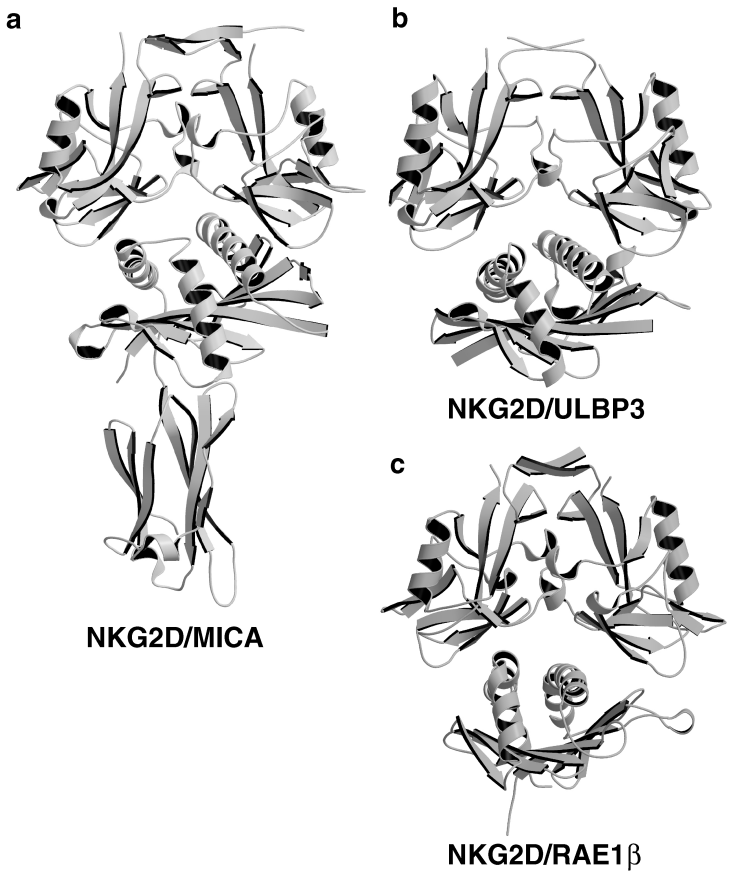

Figure 7 Structures of NKG2D in complex with (*a*) MIC-A (PDB code 1HYR), (*b*) ULBP3 (PDB code 1KCG), and (*c*) Rae-1β (PDB code 1JSK).

interaction. Detailed analysis and comparison of the hydrogen bonding networks in the complexes reveal striking differences in the topology of interacting side chains. NKG2D/MIC-A has 13 hydrogen bonds between the receptor and ligand, the largest among the three complexes; NKG2D/ULBP3 and NKG2D/Rae-1β have 9 and 7 hydrogen bonds, respectively. Three salt bridges were observed in NKG2D/ULBP3 and NKG2D/MIC-A complexes, whereas only one was observed in NKG2D/Rae-1β. Only two hydrogen bonds and one salt bridge are conserved in all three structures. Although different ligands form different hydrogen bonds with NKG2D, the signature pattern of strongest hydrogen bonds remains the same in all three complexes. Similarly, a subset of distinct but overlapping NKG2D residues participates in the hydrophobic interactions with each of the ligands and

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forms two large characteristic hydrophobic patches on the receptor surface whereby residues Tyr152, Ile182, Met184, and Tyr199 from each subunit make the largest contribution.

NK CELL RECEPTORS MEDIATE INNATE IMMUNE SURVEILLANCE

Cytotoxic T cells recognize directly viral and tumor antigens through peptide presentation by MHC molecules. To keep up with the evolving pathogenic world, T cell receptor repertoire is tuned in a receptor maturation process that modifies the TCR library to reflect current pathogens and to eliminate self-reacting T cells through gene recombination and somatic mutations. When MHC molecules present pathogenic peptides, T cells undergo clonal expansion to derive clones of monospecific T cells to stage an effective attack on viral infected or tumor cells. Although receptor maturation and clonal expansion renders cytotoxic T cells the most powerful arm of the immune system in combating infectious diseases, it is also a time-consuming process. It often takes days to activate T cell–mediated immunity upon infection. In contrast, components of the innate immune system, such as NK cells and macrophages, can often be activated in less than one hour. This fast activation kinetics puts innate immune system as the first line of immune defense. The advantage in activation kinetics, however, does not alleviate the need for NK cells to distinguish foreign versus self. The dilemma is how can NK cells retain a rapid deployment status against pathogen and yet are capable of identifying foreign and self? T cell receptors acquired this ability through an elaborate gene recombination process that is absent in NK cells. NK cell development lacks the positive/negative selection, a hallmark of T cell development.

Upon maturation, NK cells express an array of germline-encoded activating and inhibitory receptors on their surface with the distribution of each receptor largely stochastic (66). The cytolytic profile of individual NK cells depends on the balance between the activating and inhibitory receptors expressed on that particular cell. There are about 10 KIR genes in human and over 50 class I HLA molecules that are capable of presenting over 10^3 peptides. To effectively survey HLA molecules, KIR has evolved to bind multiple HLA ligands. Structurally, KIR recognition of multiple HLA allotypes is accomplished by the receptor through binding the conserved HLA residues in the polymorphic region of HLA $\alpha 1$ and $\alpha 2$ helices. While multiple ligand recognition enables KIR to monitor more class I MHC alleles, it also has the tendency to decrease the sensitivity of immune surveillance, the ability to respond to the change in the expression of a few alleles of HLA rather than to the global change in HLA expression level. The dilemma is how to maintain the ability to recognize multiple class I MHC alleles yet still display high specificity for individual MHC alleles.

A comparison between KIR2DL2/HLA-Cw3 and KIR2DL1/HLA-Cw4 complexes showed a conserved interface. Thirteen of the 17 KIR interface residues

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and 11 of the 12 HLA interface residues are conserved. When the structure of the complexes was superimposed, no large conformational changes at the interface were observed. Furthermore, no significant conformational changes were observed when the structure of ligand-free KIR2DL2 was compared with that of HLA-Cw3-bound receptor. This suggests that KIR binds HLA in a lock-and-key form of recognition. The lack of conformational variation in KIR/HLA recognition is also consistent with the fast binding kinetics as measured by BIACORE (45, 48). In contrast, the activating receptor NKG2D appears to adopt an induced-fit mechanism to recognize its ligands. The induced-fit recognition of NKG2D is also consistent with a slower binding kinetics between the receptor and its ligands than that between KIR and HLA (7, 58). Furthermore, the binding affinity between NKG2D and its ligands appears to be much higher than that between the inhibitory KIR and HLA. It is important to emphasize that both KIR and NKG2D recognize multiple ligands. However, in contrast to KIR, NKG2D ligands show low degree of sequence similarity. This suggests that KIR and NKG2D use different structural mechanism for their ligand recognition. KIR binds HLA in a lock-and-key mode but with low affinity, whereas NKG2D binds its ligands in an induced-fit mode with higher affinity. The combination of lock-and-key recognition and low-affinity binding predicts that the KIR/HLA recognition is less tolerant of mutational changes than the induced-fit, high-affinity binding of NKG2D.

Receptor-ligand interface mutations have been created to address the issue of recognition tolerance. Five charge mutations of KIR, including K44M, Y105A, E106A, D135H, and D183A, were generated, and binding affinity between the mutant KIR and HLA-Cw3 was measured by BIACORE. Four of the five single mutants resulted in complete loss of the HLA-Cw3 binding with the dissociation constant $K_D > 400\ \mu M$. The binding of E106A to the ligand is six times lower than that of the wild-type receptor. To test the mutational tolerance of NKG2D recognition, four interface charge mutations of ULBP3 were generated, H21A, E76A, R82M, and D169A. All four retained measurable binding to NKG2D, although the individual affinity varies between 30 and 230 μM. The mutational results further support the idea that the inhibitory KIR displays greater ligand specificity than NKG2D. Indeed, despite a better than 90% sequence identity between HLA-Cw1, 3, 7, 8 and HLA-Cw2, 4, 6, 15, no cross recognition was observed between their receptors, KIR2DL2 and KIR2DL1. Furthermore, the HLA allotype specificity between KIR2DL1 and KIR2DL2 appears to be controlled by a single hydrogen bond.

As to the function that these receptors play in innate immunity, the exquisite ligand specificity of KIR indicates that the inhibitory receptors sense small changes in HLA expression on cell surface and detect the loss of a few MHC alleles rather than the global loss of MHC, which only occurs in a catastrophic event. This sensitivity is particularly important because viruses often selectively downregulate the expression of a few class I MHC alleles in order to evade T cell recognition through peptide presentation. In contrast, the function of NKG2D is to recognize and kill tumor or virally infected cells through its ligand recognition. The diversity

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in NKG2D ligands presumably reflects the diversity of the pathogenic world. Encoded by a single gene, NKG2D must have the ability to recognize diverse ligands and to prevent possible pathogen-induced ligand escape. High-affinity ligand binding combined with induced-fit recognition offers a structural solution to the function of NKG2D.

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